Please replace the paragraph beginning at page 10, line 19, with the following rewritten paragraph:

B3

The invention also provides methods of treating a cell to stabilize a target polypeptide of ubiquitin protein ligase by contacting the cell with a preparation containing an effective amount of an organic compound which can competively inhibit interaction of the target polypeptide with the ubiquitin protein ligase. In preferred embodiments, the organic compound is a peptide or peptidomimetic, preferably one which is a competitive inhibitor of a WD domain, and most preferably one which includes the general chemical strucutre specified by the formula: G-H-X (3-6)-h-X-X-h-X-r-X-t (2-3)-p-X-h-h-X-X-X-X-D-X-X-X-X-h-W-D (SEQ ID No. 14).

Please replace the paragraph beginning at page 40, line 27 and continuing through page 41, line 15, with the following rewritten paragraph:

B3

The molecular marker of the test polypeptide is meant to facilitate identification of a target polypeptide interaction domain and isolation of its encoding nucleic acid. In the yeast two-hybrid embodiment of the target polypeptide trap invention, the molecular marker is typically a transcriptional activation domain which functions in yeast and which is a component of the second hybrid gene. It is understood that the second hybrid gene of the present invention can encode any of a number of alternative transcriptional activation domains including the GAL4 transcriptional activation domain region II, the strong transcriptional activator VP16, the weak synthetic transcriptional activators B17 and B112, or the amphipathic helix domain described in Giniger and Ptashne ((1987) Nature 330:670). Modifications of the transcriptional activation can be particularly useful when attempting to either increase or decrease the sensitivity of the target polypeptide trap screen. In the method of the present invention the second hybrid gene may further contain, in addition to a transcriptional activation domain, an optional nuclear localization sequence, such as that of the SV40 Large T antigen encoded by the amino acid sequence PPKKKRKVA (SEQ ID No. 13), which allows for the requisite partitioning of the product of the second hybrid gene in cases where the prey moiety is normally exclusively cytoplasmic. The second hybrid gene may additionally contain an epitope tag, such as hemagluttinin or FLAG, so that production of full length second hybrid gene prey products can be confirmed in a Western

B3 cont'd

blot. Furthermore, as explained below, the prey epitope tag provides a convenient means of testing for covalent linkage of the bait and prey moieties as is anticipated in some applications of the method of this invention. This determination is conveniently made by means of a Western blot analysis and provides a biochemical means of classifying the clones obtained from a target polypeptide trap screen.

Please replace the paragraph beginning at page 59, line 5, with the following rewritten paragraph:

In certain embodiments, the inhibitor has a molecular weight of less than 10,000 atomic mass units (amu), more preferably less than 7500 amu, 5000 amu, and even more preferably less than 3000 amu. For instance, the ubiquitin ligase/target polypeptide inhibitor can be either a peptide or peptidomimetic, preferably corresponding in length to a 3-25 mer, e.g., and in certain preferred embodiments, containing a core sequence corresponding to a WD repeat conserved sequence of G-H-X $^{(3-6)}$-h-X-X-h-X-r-X-t $^{(2-3)}$-p-X-h-h-X-X-X-X-D-X-X-X-X-h-W-D (SEQ ID No. 14); wherein "X" indicates any amino acid residue, the number ranges indicated in superscript indicate a variable number of the indicated residue type at that position, "h" indicates a hydrophobic residue, "r" indicates an aromatic amino acid residue, "t" indicates an amino acid residue which stabilizes a tight polypeptide backbone turn such as glycine, proline, aspartic acid or asparagine, and p indicates a polar amino acid residue. In other embodiments the WD repeat competitive inhibitor is provided as a gene construct for expressing the WD repeat peptide. The WD repeat peptide, peptidomimetic or gene construct is formulated in the pharmaceutical preparation for delivery to an animal to be treated.

B4

Please replace the paragraph beginning at page 60, line 15 and continuing to page 61, line 2, with the following rewritten paragraph:

B5

It is understood that the inhibitors of the invention can be competitive or noncompetitive, and can be general to a class of ubiquitin protein ligase or specific to a particular cellular target protein or somewhat broader in specificity to include multiple cellular protein targets. In

preferred embodiments, the present invention provides a peptide, or peptidomimetic that inhibits the ubiqitin-dependent degradation of the target polypeptide. The peptide/ peptidomimetic can, in certain preferred embodiments, range in size from 3-25 amino acid residues. In certain embodiments, a WD repeat inhibitor of the present invention includes a WD repeat core structure having the formula: G-H-X $^{(3-6)}$-h-X-X-h-X-r-X-t $^{(2-3)}$-p-X-h-h-X-X-X-X-D-X-X-X-X-h-W-D (SEQ ID No. 14), wherein:

G represents a glycine residue, or an analog thereof;

H represents a histidine residue, or an analog thereof;

D represents an aspartic acid residue, or an analog thereof;

W represents a tryptophan residue, or an analog thereof;

and "X" indicates any amino acid residue, "h" indicates a hydrophobic residue, "r" indicates an aromatic amino acid residue, "t" indicates an amino acid residue which stabilizes a tight polypeptide backbone turn such as glycine, proline, aspartic acid or asparagine, and p indicates a polar amino acid residue. While the invention includes all of the groups of inhibitors set forth above, the following descriptions are illustrative of an exemplary WD repeat ubiquitin protein ligase competitive inhibitor of the invention. It is understood that chemical design and other methods described for the WD repeat inhibitor apply broadly to all classes of inhibitor discussed herein.

Please replace the paragraph beginning at page 68, line 30 and continuing through page 69, line 4, with the following rewritten paragraph:

In a representative embodiment of this method, the amino acid sequences for a population of WD motifs are aligned, preferably to promote the highest homology possible. Amino acids which appear at each position of the aligned sequences are selected to create a degenerate set of combinatorial sequences. To illustrate, multiple WD repeat containing proteins are aligned and, based on these alignments, combinatorial libraries can be generated representing WD repeat

peptides which have an amino acid sequence that includes a WD core sequence represented by the formula:

B6 ON4

G-H-X $^{(3-6)}$-h-X-X-h-X-r-X-t $^{(2-3)}$-p-X-h-h-X-X-X-X-D-X-X-X-X-h-W-D (SEQ ID No. 14).

Please replace the paragraph beginning at page 72, line 27 and continuing through page 73, line 8, with the following rewritten paragraph:

While not wishing to be bound by any particular theory, it is noted that hydrophilic polypeptides may be also be physiologically transported across the membrane barriers by coupling or conjugating the polypeptide to a transportable peptide which is capable of crossing the membrane by receptor-mediated transcytosis. Suitable internalizing peptides of this type can be generated using all or a portion of, e.g., a histone, insulin, transferrin, basic albumin, prolactin and insulin-like growth factor I (IGF-I), insulin-like growth factor II (IGF-II) or other growth factors. For instance, it has been found that an insulin fragment, showing affinity for the insulin receptor on capillary cells, and being less effective than insulin in blood sugar reduction, is capable of transmembrane transport by receptor-mediated transcytosis and can therefor serve as an internalizing peptide for the subject transcellular peptides and peptidomimetics. Preferred growth factor-derived internalizing peptides include EGF (epidermal growth factor)-derived peptides, such as CMHIESLDSYTC (SEQ ID No. 15) and CMYIEALDKYAC (SEQ ID No. 16); TGF- beta (transforming growth factor beta)-derived peptides; peptides derived from PDGF (platelet-derived growth factor) or PDGF-2; peptides derived from IGF-I (insulin-like growth factor) or IGF-II; and FGF (fibroblast growth factor)-derived peptides.

B7

Please replace the paragraph beginning at page 73, line 23, with the following rewritten paragraph:

A particularly preferred pH-dependent membrane-binding internalizing peptide in this regard is aa1-aa2-aa3-EAALA(EALA)4-EALEALAA-amide (SEQ ID No. 17), which represents

B8

a modification of the peptide sequence of Subbarao et al. (Biochemistry 26:2964, 1987). Within this peptide sequence, the first amino acid residue (aa1) is preferably a unique residue, such as cysteine or lysine, that facilitates chemical conjugation of the internalizing peptide to a targeting protein conjugate. Amino acid residues 2-3 may be selected to modulate the affinity of the internalizing peptide for different membranes. For instance, if both residues 2 and 3 are lys or arg, the internalizing peptide will have the capacity to bind to membranes or patches of lipids having a negative surface charge. If residues 2-3 are neutral amino acids, the internalizing peptide will insert into neutral membranes.

B8 cont.

Please replace the paragraph beginning at page 74, line 31 and continuing through page 75, line 4, with the following rewritten paragraph:

B9

An exemplary accessory moiety in this regard would be a peptide substrate for N-myristoyl transferase, such as GNAAAARR (SEQ ID No. 18) (Eubanks et al., in: Peptides. Chemistry and Biology, Garland Marshall (ed.), ESCOM, Leiden, 1988, pp. 566-69) In this construct, an internalizing peptide would be attached to the C-terminus of the accessory peptide, since the N-terminal glycine is critical for the accessory moiety's activity. This hybrid peptide, upon attachment to a WD peptide or peptidomimetic at its C-terminus, is N-myristylated and further anchored to the target cell membrane, e.g., it serves to increase the local concentration of the peptide at the cell membrane.

Please replace the paragraph beginning at page 75, line 23, and continuing through page 76, line 2, with the following rewritten paragraph:

B10

In another embodiment of this aspect of the invention, an accessory peptide can be used to enhance interaction of the WD peptide or peptidomimetic with the target cell. Exemplary accessory peptides in this regard include peptides derived from cell adhesion proteins containing the sequence "RGD", or peptides derived from laminin containing the sequence CDPGYIGSRC (SEQ ID No. 19). Extracellular matrix glycoproteins, such as fibronectin and laminin, bind to cell surfaces through receptor-mediated processes. A tripeptide sequence, RGD, has been

identified as necessary for binding to cell surface receptors. This sequence is present in fibronectin, vitronectin, C3bi of complement, von-Willebrand factor, EGF receptor, transforming growth factor beta , collagen type I, lambda receptor of E. Coli, fibrinogen and Sindbis coat protein (E. Ruoslahti, Ann. Rev. Biochem. 57:375-413, 1988). Cell surface receptors that recognize RGD sequences have been grouped into a superfamily of related proteins designated "integrins". Binding of "RGD peptides" to cell surface integrins will promote cell-surface retention, and ultimately translocation, of the polypeptide.

B10 CON't.

Please replace the paragraph beginning at page 76, line 13 and continuing to page 77, line 15, with the following rewritten paragraph:

In an exemplary embodiment, a WD peptide or peptidomimietic is engineered to include an integrin-binding RGD peptide/SV40 nuclear localization signal (see, for example Hart SL et al., 1994; J. Biol. Chem.,269:12468-12474), such as encoded by the nucleotide sequence provided in the Nde1-EcoR1 fragment:
catatgggtggctgccgtggcgatatgttcggttgcggtgctcctccaaaaaagaagagaaag-gtagctggattc (SEQ ID No. 20), which encodes the RGD/SV40 nucleotide sequence:
MGGCRGDMFGCGAPP-KKKRKVAGF (SEQ ID No. 21). In another embodiment, the protein can be engineered with the HIV-1 tat(1-72) polypeptide, e.g., as provided by the Nde1-EcoR1
fragment:catatggagccagtagatcctagactagagccc-tggaagcatccaggaagtcagcctaaaactgcttgtaccaattgctattg taaaaagtgttgctttcattgccaagtttgtttcataacaaaagcccttggcatctcctatggcaggaagaagcggagacagcgacgaagacc tcctcaaggcagtcagactcatcaagtttctctaagtaagcaaggattc (SEQ ID No. 22), which encodes the HIV-1 tat(1-72) peptide sequence:
MEPVDPRLEPWKHPGSQPKT-ACTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRRP PQGSQTHQVSLSKQ (SEQ ID No. 23). In still another embodiment, the fusion protein includes the HSV-1 VP22 polypeptide (Elliott G., O'Hare P (1997) Cell, 88:223-233) provided by the Nde1-EcoR1 fragment:

B11

cat atg acc tct cgc cgc tcc gtg aag tcg ggt ccg cgg gag gtt ccg cgc gat gag tac gag gat ctg tac tac acc ccg tct tca ggt atg gcg agt ccc gat agt ccg cct gac acc tcc cgc cgt ggc gcc cta cag aca cgc tcg

cgc cag agg ggc gag gtc cgt ttc gtc cag tac gac gag tcg gat tat gcc ctc tac ggg ggc tcg tca tcc gaa gac gac gaa cac ccg gag gtc ccc cgg acg cgg cgt ccc gtt tcc ggg gcg gtt ttg tcc ggc ccg ggg cct gcg cgg gcg cct ccg cca ccc gct ggg tcc gga ggg gcc gga cgc aca ccc acc acc gcc ccc cgg gcc ccc cga acc cag cgg gtg gcg act aag gcc ccc gcg gcc ccg gcg gcg gag acc acc cgc ggc agg aaa tcg gcc cag cca gaa tcc gcc gca ctc cca gac gcc ccc gcg tcg acg gcg cca acc cga tcc aag aca ccc gcg cag ggg ctg gcc aga aag ctg cac ttt agc acc gcc ccc cca aac ccc gac gcg cca tgg acc ccc cgg gtg gcc ggc ttt aac aag cgc gtc ttc tgc gcc gcg gtc ggg cgc ctg gcg gcc atg cat gcc cgg atg gcg gcg gtc cag ctc tgg gac atg tcg cgt ccg cgc aca gac gaa gac ctc aac gaa ctc ctt ggc atc acc acc atc cgc gtg acg gtc tgc gag ggc aaa aac ctg ctt cag cgc gcc aac gag ttg gtg aat cca gac gtg gtg cag gac gtc gac gcg gcc acg gcg act cga ggg cgt tct gcg gcg tcg cgc ccc acc gag cga cct cga gcc cca gcc cgc tcc gct tct cgc ccc aga cgg ccc gtc gag gaa ttc (SEQ ID No. 24)

B11 cont.

which encodes the HSV-1 VP22 peptide having the sequence:

MTSRRSVKSGPREVPRDEYEDLYYTPSSGMASPDSPPDTSRRGALQTRSRQRGEVRFVQ
YDESDYALYGGSSSEDDEHPEVPRTRRPVSGAVLSGPGPARAPPPPAGSGGAGRTPTTAP
RAPRTGRVATKAPAAPAAETTRGRKSAQPESAALPDAPASTAPTRSKTPAQGLARKLHF
STAPPNPDAPWTPRVAGFNKRVFCAAVGRLAAMHARMAAVQLWDMSRPRTDEDLNE
LLGITTIRVTVCEGKNLLQRANELVNPDVVQDVDAATATRGRSAASRPTERPRAPARSA
SRPRRPVE (SEQ ID No. 25).

Please replace the paragraph beginning at page 77, line 16, with the following rewritten paragraph:

In still another embodiment, the fusion protein includes the C-terminal domain of the VP22 protein from, e.g., the nucleotide sequence (Nde1-EcoR1 fragment):

B12 cat atg gac gtc gac gcg gcc acg gcg act cga ggg cgt tct gcg gcg tcg cgc ccc acc gag cga cct cga gcc cca gcc cgc tcc gct tct cgc ccc aga cgg ccc gtc gag gaa ttc (SEQ ID No. 26)

which encodes the VP22 (C-terminal domain) peptide sequence:
MDVDAATATRGRSA-ASRPTERPRAPARSASRPRRPVE (SEQ ID No. 27)

In certain instances, it may also be desirable to include a nuclear localization signal as part of the WD peptide.

B12 cont.

In the generation of fusion polypeptides including the subject WD peptides, it may be necessary to include unstructured linkers in order to ensure proper folding of the various peptide domains, and prevent steric or other inteference of the heterologous domains with the PV inhibitory activity of the WD peptide. Many synthetic and natural linkers are known in the art and can be adapted for use in the present invention, including the (Gly3Ser)4 (SEQ ID No. 28) linker.

Please replace the paragraph beginning at page 80, line 17, with the following rewritten paragraph:

B13

The pseudodipeptide is then coupled at the C-terminus, according to the above example, with a suitably protected tyrosine residue, and at the N-terminus with a protected alanine residue, by standard techniques, to yield the protected tetrapeptide isostere A-I-Y-Y (SEQ ID No. 29). The terapeptide is then further condensed with the olefinic tripeptide analog derived by similar means for Lys-Ala-Arg, and so forth to build up the full WD peptide. The protecting groups are then removed with strong acid to yield the desired peptide analog, which can be further purified by HPLC.

Please replace the paragraph beginning at page 142, line 29 and continuing to page 144, line 10, with the following rewritten paragraph:

B14

S. cerevisiae INVSc1 (Invitrogen) and Y81 cells (gifts of S. Elledge) were used to assess the *in vivo* degradation of the papillomavirus E2 or pRB by the engineered Cdc4p ubiquitin-protein ligases. The human osteosarcoma Saos-2 cell was a gift of Drs. David Thomas and Philip Hinds. The galactose inducible pRB expression plasmid p2202TRB was a gift of Dr. Robert Weinberg (Hatakeyama et al. (1994) Genes Dev. 8: 1759-71). The engineered Cdc4p derivatives designed for targeting pRB degradation were constructed as follows: $Cdc4p^{F/WD}$-LTP and $Cdc4p^{F/WD}$-LTP(FGSK) cDNAs were generated by PCR using the 5' primer 5'-

GCGGATCCACCATGGATAAMAAAGAGGGACCTAATAAC-3' (SEQ ID No. 30) that hybridizes to *CDC4* corresponding to residues 270 to 277 with a BamHI site and the ATG translation initiation codon at the 5' end, and the 3' primer encoding sequences of either the Cdc4p C-terminus (residues -779) in frame fused to the pRB binding domain of the SV40 large T antigen (residues 103-115), or the same primer carrying point mutations of the LFCSE (SEQ ID No. 47) motif that abolishes its interaction with pRB (DeCaprio et al. (1989) Cell 58:1085-95; Dyson et al. (1989) Science 243: 934-7; Figge et al. (1993) Protein Sci 2: 155-64; Munger et al. (1989) EMBO J 8: 4099-105); Yang et al. (1995) Nucleic Acids Res 23: 1152-6). The sequences of these two 3' primers carrying the NotI site and a translation stop codon are 5'-GCGCGGCCGCCTACTCATCATCACTAGATGGCAMCTTCTGAGCAAAACAG

CCCTCTGG TATTATAGTTGTCCTCGT-3' (SEQ ID No. 31) and 5'-CGCGGCCGCCTACTCATCATCACTAG ATGGCAMTTGAGCCAAAGTTTTCTCTGGTATTATAGTTGTCCTCGT-3' (SEQ ID No. 32). The resulting PCR fragments encoding Cdc4p$^{F/WD}$-LTP or Cdc4p$^{F/WD}$-LTP(FGSK) hybrids were digested with BamHI and NotI, and were subsequently cloned into the pYES2 vector (Invitrogen) for expression in Y81 cells under the control of the *GAL1,10* promoter. The Cdc4p$^{F/WD}$-E7N or Cdc4p$^{F/WD}$-E7N(DLYC) hybrid constructs were obtained by a two-step PCR approach as described in detail (Dieffenbach, 1995). The first PCR reactions for making individual Cdc4p$^{F/WD}$-E7N or E7N(DLYC) DNA fragments were conducted using pYES-F-CDC4 (this work), pGST-E7(2-35) or pGST-E7(2-35)(DLYC) plasmids as templates (Gifts of Dr. K. Munger). The sequences for the primer sets used are as follows: 5'-

GCWATCCACCATGGATAATITAAAGAGGGACCTAATAAC-3' (SEQ ID No. 33) (5'-extreme) and

5'-GTAGGTGTATCTCCATGTGGTATrATAGTrGTCC-3' (SEQ ID No. 34) for Cdc4p$^{F/WD}$, 5'-

GGACAACTATAATACCACATGGAGATACACCTAC-3' (SEQ ID No. 35) and 5'-

GCCTCGAGTCACTCCTCCTCTGAGCTGTC-3' (SEQ ID No. 36) (3'-extreme) for E7N or E7N(DLYC), respectively. The second step PCR reactions were conducted using the same 5'- and 3' extreme primers to ligate Cdc4p$^{F/WD}$/E7N or Cdc4p$^{F/WD}$/E7N(DLYC) PCR fragments

together. The resulting hybrid Cdc4p$^{F/WD}$-E7N or Cdc4p$^{F/WD}$-E7N(DLYC) DNA fragments were digested with BamHI and XhoI which were introduced by the 5'- and 3'- extreme primers, respectively, and were cloned into the p426-ADH plasmid (ATCC) for constitutive expression in Y81 cells under the control of the ADH promoter. Cdc4p$^{F/WD}$-ElC were constructed similarly by the two-step PCR approach using primer pairs 5'-GCGGATCCACCATGGATAAMAAAGAGGGAC CTAATAAC-3' (SEQ ID No. 37) (5'-extreme)-3' and 5'-CCTATCACATCTATATTTTATTGGTATTA TAGTTGTC-3' (SEQ ID No. 38) for Cdc4p$^{F/WD}$, and 5'- GACAACTATAATACCAATAAAATATAG ATGTGATAGG-3' (SEQ ID No. 39) and 5'-GCCTCGAGTCATAATGTGTTAGTATTTTGTCCTG-3' (SEQ ID No. 40) for E1C. The resulting Cdc4p$^{F/WD}$-EIC fragment was cloned into the BamHI/XhoI sites of p426-ADH vector. EE-tagged HPV16-E2 and HPV16-E2(E39A) were generated by PCR using primers 5'-GCGGATCCACCATGGAGGAAGAAGAGTATATGCCCA TGGAGGAGACTCTT TGCCAACGTTTTAAATGTG-3' (SEQ ID No. 41) and 5'-GCGCGGCCGCTCATATAGACATAAATCCA GTAGAC -3' (SEQ ID No. 42), and the resulting PCR fragments were cloned into the single copy plasmid pCM185 for expression under the control of the tetracycline-repressible (tetO^{R}) promoter in *S. cerevisiae*.

Please replace the paragraph beginning at page 144, line 11, with the following rewritten paragraph:

βTrCp-E7N and βTrCP-E7N(DLYC) were also constructed by the two step PCR approach with primer pairs 5'-GCGGATCCGCCACCATGGACTACAAGGACGAC GATGACAAAGATGACCCGGCCGAGGCGGTGCTG-3' (SEQ ID No. 43) and 5'-GTAGGTGT ATCTCCATGTCTGGAGATGTAGGTGTATG-3' (SEQ ID No. 44) for PTRCP, 5'-CATACACCTA CATCTCCAGACATGGAGATACACCTAC-3' (SEQ ID No. 45) and 5'-GCGCGGCCGCTCACTCCTCCTCTGAGCTGTC-3' (SEQ ID No. 46) primer sets for E7N or E7N(DLYC). The final PCR fragments were cloned into the BamHI/NotI sites of pcDNA3 (Invitrogen). The cloned PCR fragments described above were sequenced by the BCMP core facility at Harvard Medical School. Expression of the engineered Cdc4p hybrids in Y81 cells were confirmed by immunoblotting using the anti-Cdc4p polyclonal antibody.